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Protein translocation: **Delivering virulence into the host cell**

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A wide variety of plant and human bacterial pathogens use a specialized 'type III' protein secretion system to deliver virulence factors into host cells. Appendage-like surface structures have recently been identified on several bacterial pathogens and there are indications that these may be conduits for virulence factor delivery.

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Pathogens generally produce specialized proteins that are required for, or enhance, their virulence towards a host organism. Most such 'virulence factors' are found on the surface of pathogenic organisms or are secreted into the environment. Pathogenic bacteria must therefore have mechanisms to release and deliver virulence factors. Often these proteins are injected into the host cell, where they cause a wide variety of events, including phagocytosis, anti-phagocytosis and subversion of other host signaling pathways. As it is inefficient to secrete these virulence factors into the extracellular fluid and rely on diffusion for delivery, several plant and animal bacterial pathogens have developed a specialized secretion system, called a type III secretion system, to mediate the direct transfer of proteins into host cells.

Type III secretion systems have been found in many human, animal and plant pathogens (reviewed in [1]). The secretion apparatus is complex, as exemplified by the *Yersinia* system where it is made up of more than 20 proteins. This apparatus delivers the effector molecules directly from the cytoplasm of the bacteria, through both inner and outer membranes, to the bacterial surface, and from there into the host cell. Proteins secreted through this system lack an amino-terminal signal sequence, as is characteristically present on proteins secreted by other systems. Functional conservation of the type III secretion and translocation machinery has been shown by genetic cross-complementation in *Yersinia*, *Salmonella* and *Shigella* species [2].

Many effectors of the type III secretion system that have been identified in *Yersinia*, *Shigella*, *Salmonella* and enteropathogenic *Escherichia coli* appear to act directly on intracellular host factors. Most recently it has been shown that the *Salmonella typhimurium* protein SopE is translocated into the host cell where, by activating GDP–GTP exchange in members of the Rho family of small GTPases, it induces cytoskeletal and nuclear responses that lead to bacterial entry into host cells [3].

While many type III effectors are being identified and assigned functions, one of the major questions is how these proteins are delivered into host cells. Many gene products have been identified that appear to be components of a type III secretion apparatus, but the structures and organization of these proteins are not known. Recent studies on *S. typhimurium* and enteropathogenic *E. coli* have shed some light on the structure of type III secretion systems.

Supramolecular structure of type III secretion systems

Salmonella species are a common cause of diarrhea in humans and animals. These bacterial pathogens have the ability to be taken up by cells that are not normally phagocytic, and this contributes to their virulence (reviewed in [4]). *S. typhimurium* has at least two type III secretion systems, one of which directs the translocation of several proteins into host cells, thereby promoting bacterial invasion [5]. A number of the proteins that are thought to be involved in the secretion apparatus in *S. typhimurium* show extensive sequence similarity to proteins involved in organelle assembly, such as the flagellar export machinery. This suggests that the type III translocation systems may involve some type of supramolecular surface structure.

A few years ago, it was shown by scanning electron microscopy that the presence of 'invasomes', appendages on the surface of *S. typhimurium*, correlated with expression of a type III secretion system and bacterial invasion [6]. These structures appeared upon contact of the bacteria with cultured epithelial cells; their formation did not require *de novo* protein synthesis, and they were only present until internalization into the host cell was initiated [6]. More recently it was shown that both wild-type *S. typhimurium* and a strain lacking all flagellar proteins have complex cell-surface structures resembling needles [7]. There are 10–100 of these needle complexes per cell and, while the base of the structure resembles a flagellar basal body, the needle structure — a stiff straight tube 80 nm long and 13 nm wide — is completely distinguishable from flagellar basal bodies.

Intriguingly, these needle complexes are not found in *S. typhimurium* strains with mutations in genes encoding components of type III secretion systems. Three major protein species were found to be components of the supramolecular structure and identified as InvG, PrgH

and PrgK, all known components of one of the two *S. typhimurium* type III secretion systems [7]. All three are predicted to be outer membrane proteins with sequence motifs characteristic of bacterial lipoproteins [4], and provocatively, InvG is also required for the assembly of invasomes [6]. Needle complexes isolated from a *S. typhimurium* strain expressing an epitope-tagged PrgH could be visualized by immunoelectron microscopy after labelling with an antibody against the epitope, confirming that PrgH is a component of this structure [7].

The similarity between *S. typhimurium*'s needle complex and components of the flagellar export system suggest an interesting possibility: the needle complex may act, like the flagellar basal body, as a channel through which the secreted proteins cross the two bacterial membranes [7]. The components of the transiently assembled invasomes have not been identified, but the possibility that the needle complex is the base of this structure is striking. It has yet to be shown whether the *S. typhimurium* needle-structures contact the host surface and so really act as a translocation 'tube'. However, such appendage-mediated contact has been recently demonstrated in enteropathogenic *E. coli* [8].

Appendages form bridges to host cells

Enteropathogenic *E. coli* is a major cause of infantile diarrhea. They adhere to intestinal epithelial cells and induce a rearrangement of the host cytoskeleton so as to form what are known as 'attaching and effacing' lesions. These lesions are characterized by the loss of the brush-border microvilli, and formation of pedestal-like structures on the host cell surface upon which the bacteria reside. The dramatic loss of intestinal microvilli could lead to diarrhea via malabsorption, but this mechanism has yet to be proven. This pathogen also uses a type III secretion system to exploit host cells [9].

Labelling cells with an antibody against the type III secreted protein EspA has recently revealed filamentous structures on the bacterial surface [8]. The anti-EspA-labelled structures were 50 nanometers in diameter and up to 2 micrometers long; they were seen on wild-type cells, but not in *espA* mutants or strains with mutations in genes for components of the type III secretion apparatus. The anti-EspA-labelled filaments formed bridges between the bacteria and eukaryotic host cells (Figure 1) [8]. Taken together with the evidence that EspA is required for translocation of EspB into host cells [8], these observations suggest that the EspA filaments act as channels for delivering proteins into host cells, although the mechanism of translocation is far from established.

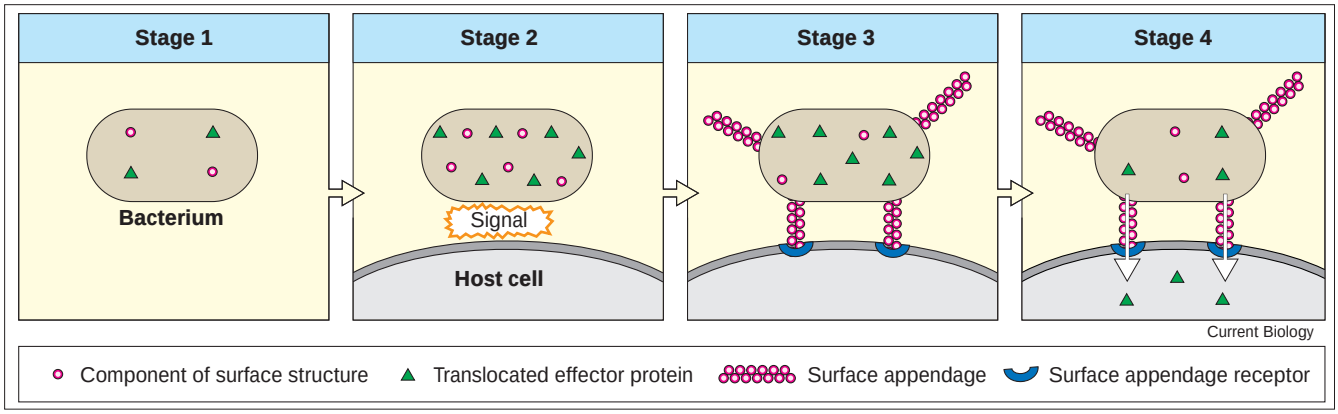
In an analogous manner to *S. typhimurium*'s invasomes [6], the EspA filaments are excluded from the region of intimate cell–cell contact when attaching and effacing lesions are formed on the host cell, and fully developed attaching and effacing lesions are devoid of EspA filaments [8]. Many similarities exist between the surface structures of *S. typhimurium* and the EspA filaments of enteropathogenic *E. coli*. The two types of structure are similar in size and shape; both are shed after they trigger changes in the host cell; and both are assembled in a type III secretion system-dependent manner. One of the interesting differences between these pathogens is that, in *S. typhimurium*, the complex is made up of components of the secretion apparatus, whereas in enteropathogenic *E. coli*, type III secreted proteins are integral to the structure. This difference could be moot — when all the components of the structures are identified, both types of proteins could turn out to be present, or the secreted proteins could play multiple roles in the bacteria.

The translocation of proteins from bacteria to inside host cells by type III secretion systems seems to be a common mechanism used by a wide variety of bacterial pathogens. A type III-dependent surface appendage has been recently discovered on the plant bacterial pathogen *Pseudomonas syringae* [10]. It has also been postulated that the surface projections and associated fimbrial extensions found on *Chlamydia* cells are this obligate intracellular pathogen's type III secretion apparatus and associated virulence effectors, respectively [11].

Figure 1

Surface appendages on enteropathogenic *E. coli* that are dependent on type III secretion. A transmission electron micrograph showing plasmid-cured enteropathogenic *E. coli* strain JPN15 immunogold-labelled with anti-EspA antiserum after a 3 hour incubation with Hep-2 cells. Before the formation of attaching and effacing lesions on the host cell, labelled filaments were seen to form a bridge between the bacteria and the Hep-2 cell surface (arrow). Scale bar = 200 nm. (Reproduced with permission from [8].)

Figure 2



A model of protein translocation from a pathogenic bacteria into host cells. The structural components of the surface appendages are already present in the bacterium before contact with a host cell (stage 1 [6]). An as yet unidentified signal triggers the assembly of the surface appendages that contact the host cell (stages 2 and 3). The surface appendage functions as a channel through which effector proteins are translocated into the host cell (stage 4) [7,8]. The surface appendages are shed after initiating changes in the host cell [6,8].

A model for protein translocation

These recent developments concerning type III secretion can be incorporated into a model for protein translocation by bacterial pathogens (Figure 2). In this model, an as yet unidentified signal triggers the assembly of the bacterial surface appendages, which then act as channels for delivery of effector proteins into host cells. Many aspects of this model need to be examined further to elucidate the structure and mechanism of type III secretion systems. In *S. typhimurium*, it is known that *de novo* protein synthesis is not required for the formation of surface appendages [6], leading to the idea that the protein subunits are already present inside the bacterium before contact with host cells. This remains to be investigated in other pathogenic bacteria. How the bacteria sense the host cell, and the nature of the trigger that signals the commencement of assembly of these appendages, are presently unknown.

The composition and assembly of the surface structures, be it of secreted proteins or apparatus components, is an area of increasing interest. While in enteropathogenic *E. coli* the EspA filaments form a bridge to the host, this remains to be shown in other pathogens. The identity of the surface appendage receptor on the host cell is also unknown. A major outstanding question is whether these surface structures are indeed channels through which effectors can be delivered directly into host cells. As it is likely that a common mechanism is used to deliver virulence factors into host cells, a clear understanding of this type III secretion and translocation system should lead to the development of ways in which to inhibit it, and therefore control many bacterial diseases.

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